



## Relationship between Beta-Lactoglobulin and Bovine Submaxillary Mucin: Structure and Tribology Studies

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**INTRODUCTION**

For food oral processing, any specific component in the food products and its structural changes in varying environment can give crucial influence on the sensory acceptance of the products. The objective of this research was to investigate the interaction between beta-Lactoglobulin (BLG), the major whey protein, and bovine submaxillary mucin (BSM), a (model) major salivary component, when mixed (1:1) at different pHs (pH 3.0, 5.0 and 7.4) in order to broaden our understanding of food oral processing on the molecular level. High and low field Nuclear Magnetic Resonance (NMR), Dynamic Light Scattering (DLS) and Circular Dichroism (CD) techniques were employed to study the structural changes. A Mini-Traction Machine (MTM) was then employed to investigate the friction and lubrication properties of the proteins at a compliant interface, as a mimic of oral processing of dairy products.

**High field-NMR results**

The possible interaction mainly takes place based on the chemical shifts between 3.1 to 4.4 ppm, mostly corresponding to polar amino acids, sulfate ester, sialic acid, and GalNAc residues. To conclude, the interaction between these two proteins is mostly of hydrophilic origin.

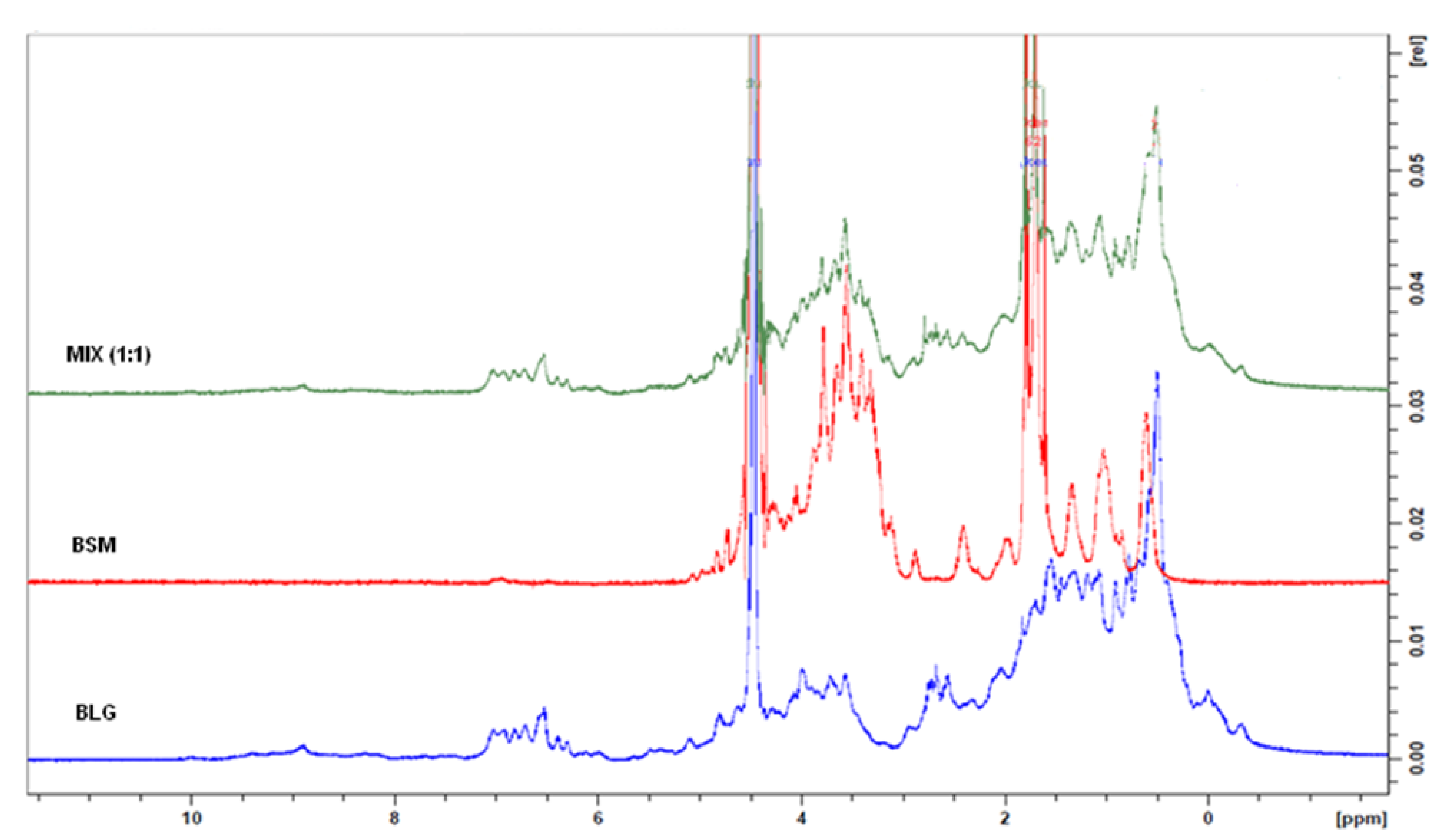
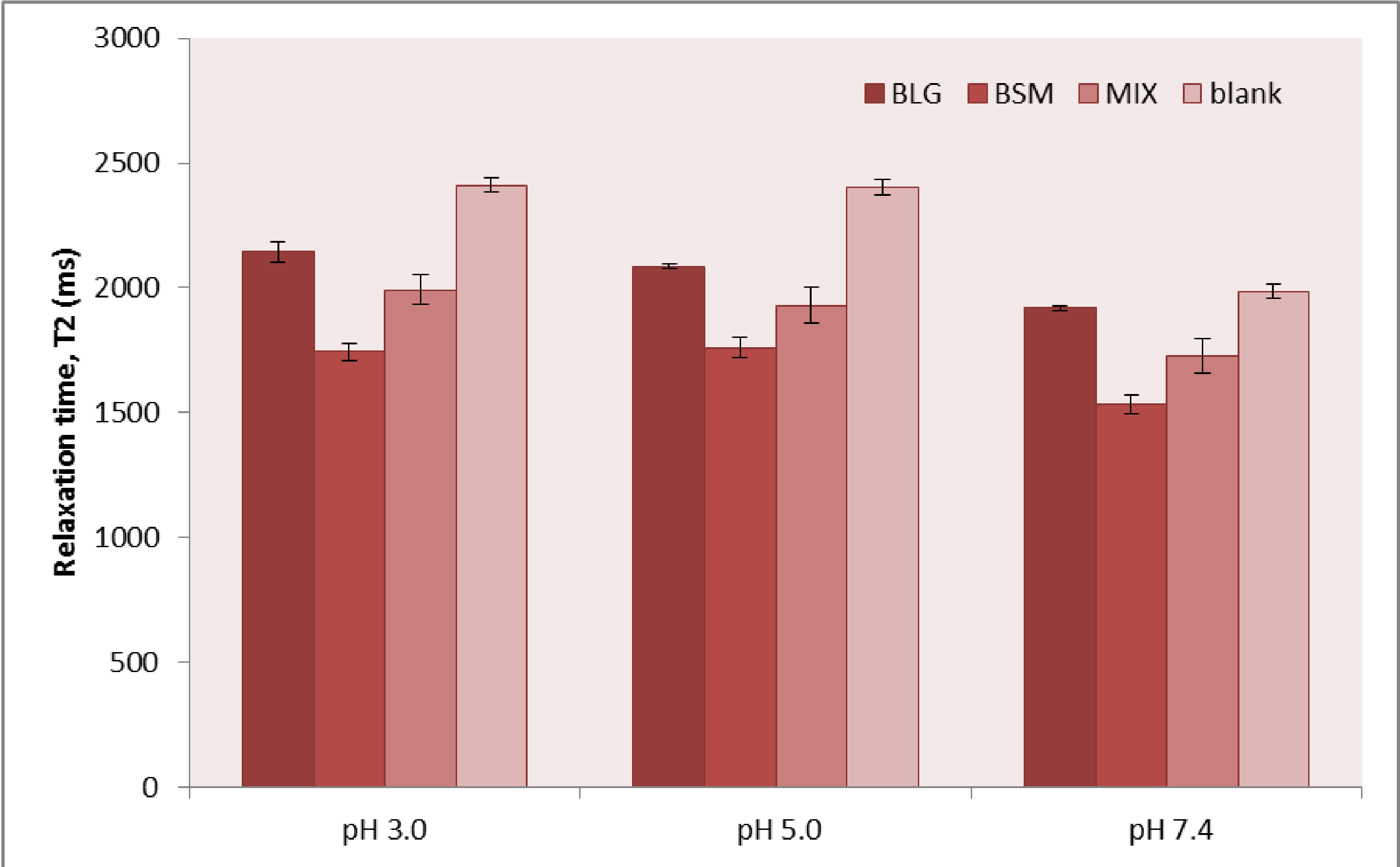


Figure 2. High field NMR spectra of BLG, BSM, and the mixture at pH 7.4.

**Low field-NMR results**

A decrease in water mobility in the mixture compared to the pure BLG and buffer solutions was possibly connected to the fewer number of hydrophilic binding sites available for water-protein interaction. The interaction between BLG and BSM also caused a more compact configuration than BSM alone and resulted in higher water mobility compared to pure BSM solution.

Figure 3. Low field NMR spectra of BLG, BSM, and the mixture at pH 3.0, 5.0, and 7.4



The present results provide insight into the interactions of the BSM protein with the BLG; further model biopolymers and methods are currently under investigation.

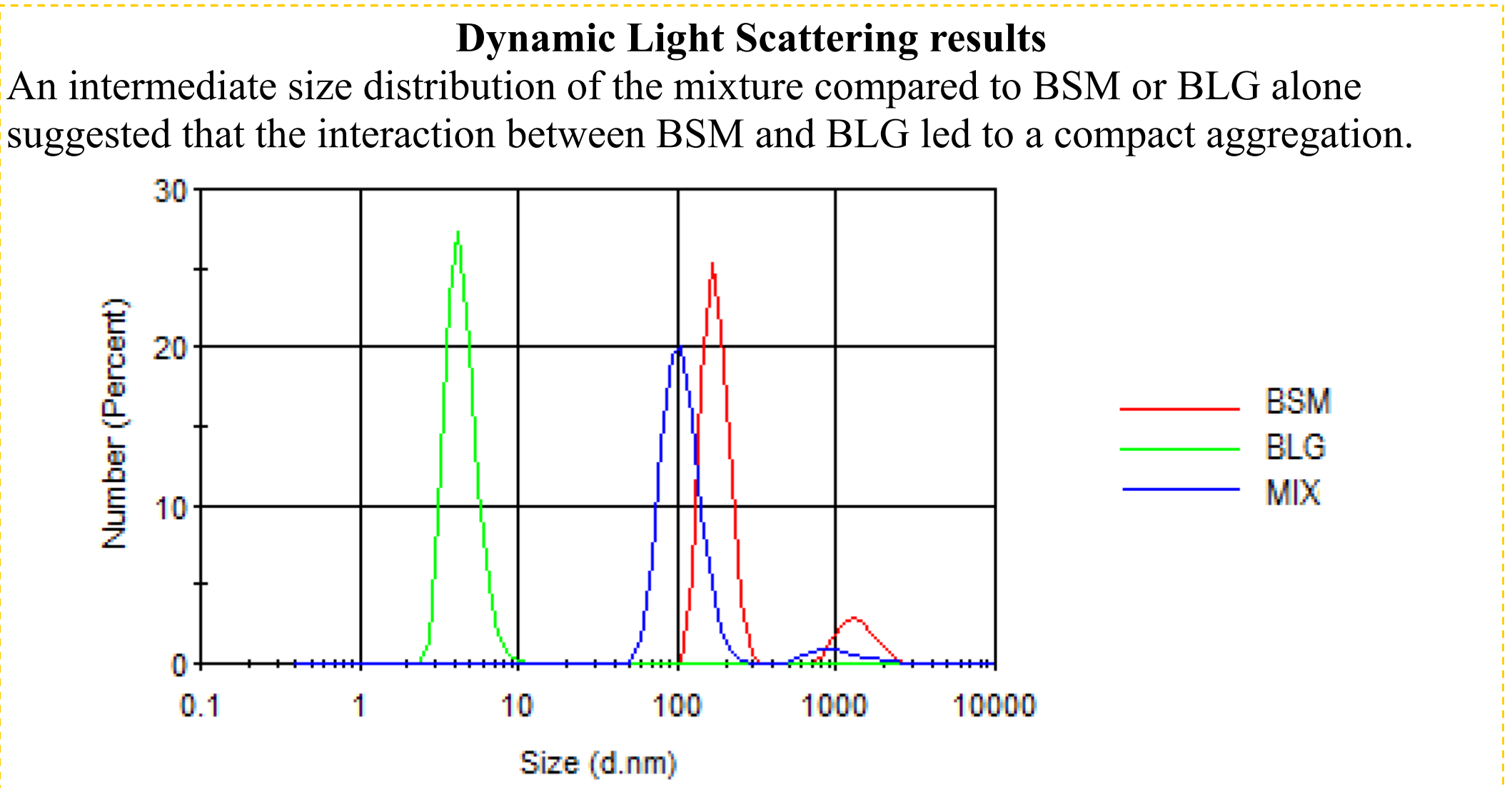


Figure 1. Size distribution curve of BSM, BLG and the mixture at pH 7.4

Table 1. Chemical shifts of the repeated amino acids and other residues in BSM molecules

Chemical shift (ppm)	Residues
0.6 – 0.9	Valine
1.2	Nonglycosylated Threonine
1.4	Alanine
1.5 – 1.8	GalNAc residues
2.1 – 2.3	Proline
2.8 – 3.1	Cysteine
3.2 – 4.4	Threonine, Serine, sulfate esters, sialic acid residues
4.6 – 4.9	GalNAc residues

Table 2. The interacted residues of BSM and BLG in their mixture (1:1)

	Residues 3.1– 4.4 ppm
BSM	Threonine
	Serine
	Sulfate ester
	Sialic acid
	Threonine
	Tryptophan
BLG	Serine
	Aspartic acid
	Tyrosine
	Asparagine
	Phenylalanine
	Cysteine
	Histidine
	Proline
	Glutamine
	Leucine

**Mini-Traction Machine results**

At all pH conditions, BSM was observed to provide the lowest friction forces. However, the relative difference compared to BLG and the BSM-BLG mixture was gradually diminishing with lowering pH. This is partly due to increasing friction forces of BSM with decreasing pH, and partly due to the complexation of BLG and BSM at low pH, where these components are oppositely charged.

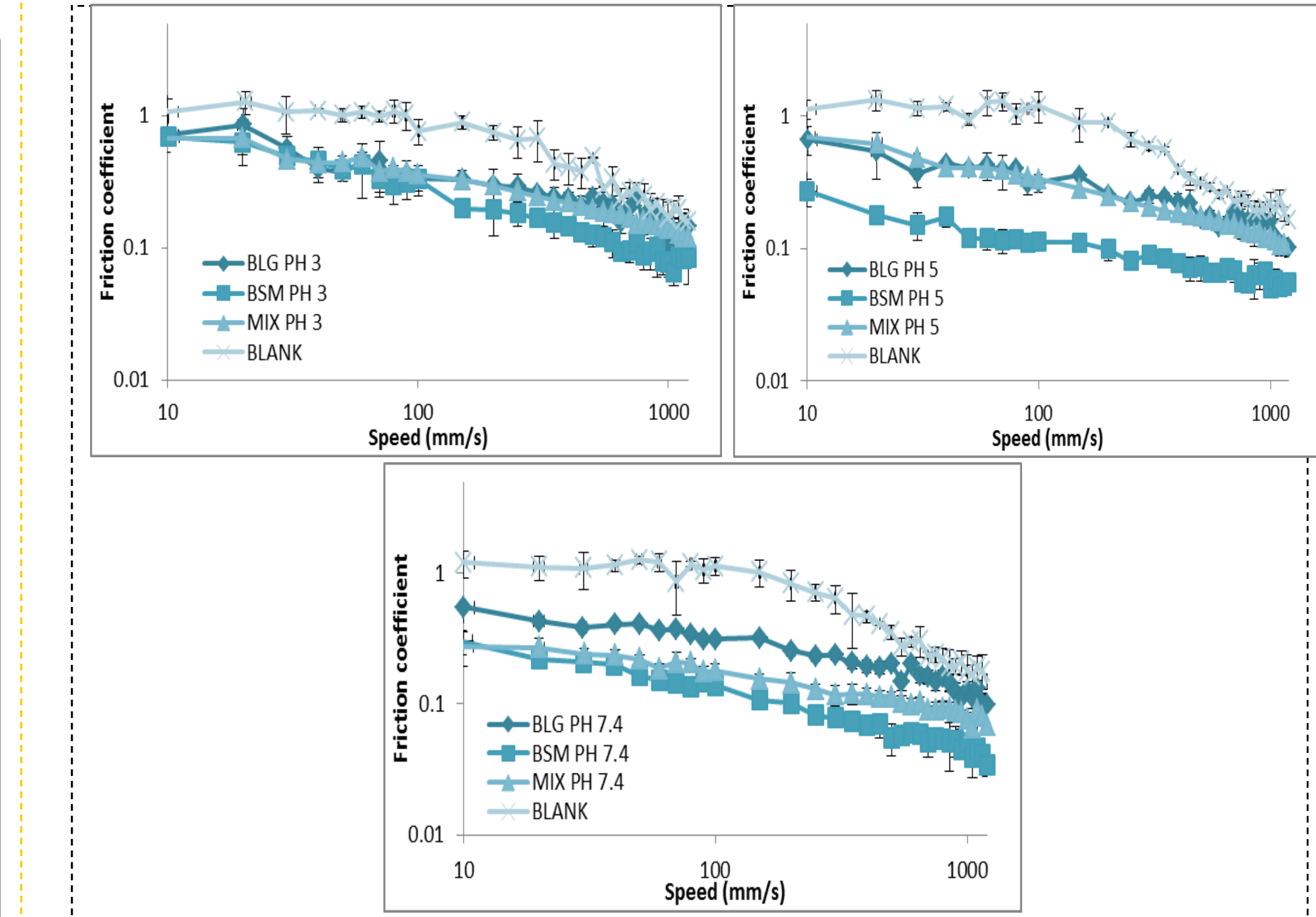


Figure 4. Friction coefficient distribution of BLG, BSM and the mixture over buffer at pH 3.0, 5.0, and 7.4

$$f(x+\Delta x)=\sum_{i=0}^{\infty}\frac{(\Delta x)^i}{i!}f^{(i)}(x)$$
$$\int_a^b \epsilon \Theta + \Omega \int \delta e^{i\pi} = \{2.7182818284\}$$
$$\chi^2 \sum \gg$$